2013-Pos Board B32

Simulation-Based, Systematic Approach to Understanding the Long-Time Dynamics of Stoichiometrically-Mismatched, Self-Assembled Protein Hydrogel Networks

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We present mesoscale simulation results and analytical derivations for the long-time equilibrium dynamics of model peptide networks assembled from mixing stoichiometrically mismatched amounts of ''junction'' and ''linker'' components. The analysis evaluates how the migration of defects controls the rate of relaxation of shear stress, and how this rate is related to junction multiplicity, defect concentration, linker stiffness, single vs. multiple junction pairings, and linker looping ability. The shear-relaxation time and viscosity are predicted to fall away sharply when junction and linker concentrations are mismatched by even 1%. The mean stress relaxation per defect migration event obtained through simulations of low valent networks with single junction pairings is from two to three times greater than assumed by standard simple theories. The rates of defect migration in networks with multiple junction pairings and/or loops are accelerated and result in lower shear relaxation times. Discounting the looped linkers from the density of active chains and treating linkers involved in multiple junction pairings as parallel springs leads to a static shear modulus (G) quadratic in the number of effective elastic chains ($N_{elastic}$) on a single master curve. Furthermore, the time-dependence of viscosity during gel ''aging'' is modeled as an approach to equilibrium through diffusion-limited recombination of complementary defects.

2014-Pos Board B33

Effects of Pressure on the Adsorption of Proteins at Aqueous-Solid Interfaces

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The spontaneous adsorption of proteins at aqueous-solid interfaces plays an important role in nature, medicine, and biotechnology. A sound understanding of the molecular mechanisms and interactions upon protein adsorption is required for the development of new materials coatings and for the choice of solvent conditions. So far, protein adsorption studies have been carried out mainly as a function of protein solution concentration, pH-value, and surface chemistry. In addition, several temperature-dependent studies have shed some light on the thermodynamics underlying protein adsorption. In contrast, effects of pressure on this process are widely unknown. Applying pressure to molecular systems generally offers access to all kinds of volume changes occurring during assembly of molecules, phase transitions, and chemical reactions. In the case of protein adsorption, using pressure as a thermodynamic variable allows for the determination of volume changes of adsorption, volume changes of unfolding in the adsorbed state, and changes of protein-interface interactions that determine the degree of protein adsorption. We have designed new high pressure cells for total internal reflection fluorescence (TIRF) spectroscopy and neutron reflectometry (NR) for pressures up to 2500 bar in order to access these quantities. The results obtained so far indicate a pressure-induced increase of the degree of protein adsorption at both the water-silica and water-poly(styrene) interfaces. Moreover, a drastic decrease of the volume change of unfolding has been found when proteins are adsorbed at the water-silica interface. Apparently, pressure exerts a distinct influence on the process of protein adsorption and provides a new complemental view on the underlying mechanism.

2015-Pos Board B34

Ionic Conductance through Fibrin Networks

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One of the body's primary mechanisms to curtail excess blood loss in the event of injury is the formation of a thrombus. This hemostatic clot is the direct result of fibrinogen's conversion to fibrin in the presences of thrombin. Fibrin network formation is strongly dependent on the amount of thrombin present during its development. In our study, we address the effect of thrombin on the distribution of the network opening sizes. Fibrin networks are grown over micron-sized pores in polymer membranes, varying attributes such as enzyme concentration, pH, etc. The networks are then characterized using optical techniques such as confocal and differential interference contrast microscopies. We present experiments that measure the ionic flow through a fibrin network grown over a pore. Polyethylene glycol (PEG) molecules suspended in solution are used to attenuate the ionic current as they pass through the fibrin network. The growth characteristics and correlated current blockade distributions will be presented.

2016-Pos Board B35

Effects of Zinc Binding and Chelation on Structures and Dynamics in Amyloid-Beta Dimers

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Amyloid-β (Aβ) is a protein of 39-43 amino acids that self-associates into a diverse array of neurotoxic aggregates linked to Alzheimer's disease (AD). Recently, Aβ dimers and trimers have been shown to cause memory deficits when they are recruited to the synapse, perhaps due to zinc release during neurotransmission. We have employed single-molecule fluorescence methods to investigate dimer structures, to understand zinc-induced structural change and determine whether it may be reversed by metal chelation. Single-pair FRET measurements were performed using Aβ40 peptides labeled at the N-termini with donor and acceptor dyes; the donor peptide was additionally labeled at the C-terminus with a Lys-biotin moiety to permit tethering to a functionalized cover slip. Time-dependent FRET efficiencies (E_{FRET}) were determined by measuring fluorescence from individual surface-tethered dimers, affording insight into dimer structures and structural dynamics. Characteristic E_{FRET} values were determined for dimers in metal-free samples and in the presence of Zn^{2+} (1 equiv.), with and without excess amounts of the zinc chelator clioquinol (CQ, 10 equiv.). Under all sample conditions, dimers appear to exhibit at least two characteristic structures, as evidenced by at least two broad peaks in each of the E_{FRET} ensemble histograms. Zinc binding causes a slight structural change that is not reversed by chelation. Strikingly, zinc also severely limits dimer structural dynamics: while 29% of dimers in metal-free samples visit two E_{FRET} values over time, only 12% of dimers in the presence of zinc exhibit structural change. Chelation does not reverse this effect, as only 9% of dimers in the presence of CQ show structural dynamics. These results lend new insights into the role(s) of zinc in Aβ association and the use of metal chelators in AD prevention and treatment.

2017-Pos Board B36

Understanding Prion Aggregation in Amyloids by Analyzing their Mechanical Properties using AFM

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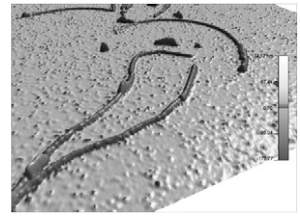
Prion diseases such as Creutzfeldt-Jakob or mad cow diseases originate from the misfolding and aggregation of proteins, with invariably lethal outcome. In this study we generate prion fibrils and investigate their structural properties by atomic force microscopy. Fibril shapes are analyzed statistically using worm-like chain models that describe the bending of semi-flexible polymers under thermal fluctuations. This way we quantify the mechanical properties of wild-type and mutant prion fibrils exhibiting highly diverse morphologies characterized by distinct bending rigidities and breakability. Acquiring such structural insights on prion misfolded forms will provide the appropriate tools to test experimentally the hypothesis of a link between the conformational stability of prion protein fibrils and their propensity to be toxic and infectious.



2018-Pos Board B37

Visualization of the Dynamics of Fibrin Clot Growth One Molecule at a Time by Total Internal Reflection Fluorescence Microscopy

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Individual fluorescently-labeled fibrin(ogen) molecules and their assembly to make a clot were observed by total internal reflection fluorescence microscopy (TIRFM). We used the bleaching of the fluorescent labels to determine the number of active fluorophores attached non-specifically to each molecule. From the total intensity of bleaching steps, as single-molecule signature events, and the distribution of active labeling, we developed a new single-molecule intensity calibration which accounts for all molecules, including those ''not seen''. Live observation of fibrin polymerization in TIRFM by diffusive mixing of thrombin and plasma revealed the real-time growth kinetics of individual fibrin fibers quantitatively at molecular level. Some fibers thickened in time to thousands of molecules across equivalent to hundreds of nm in diameter, whereas others reached an early stationary state at smaller diameters. This new approach to determine the molecular dynamics of fibers growth provides information important for understanding clotting mechanisms and the associated clinical implications.